EFFECTS OF REPELLENTS ON MOSQUITO BEHAVIOR

A.A. KHAN
Department of Entomology
University of Alberta

Quaestiones entomologicae
1:1 – 35. 1965

The behavior of Aedes aegypti *L. and other species of* Aedes *in relation to repellent chemicals was studied. The repellents used were dimethyl phthalate, ethyl hexanediol, N, N-diethyl metatoluamide and indalone. The effect of these repellents on the behaviour of mosquitoes was studied firstly by placing the repellents on selected parts of the environment and secondly by painting them on parts of mosquitoes themselves where chemoreceptors are known to occur, such as the antennae, labium, and tarsi. The aspects of behavior studied were: feeding on blood and on sugars, mating, oviposition, the reactions to wind, geotaxis and orientation to centrifugal force, and the visual response to black stripes. All these aspects of behavior are affected significantly by repellents. Dimethyl phthalate has the greatest effect of the four repellents on blood feeding behavior when they are painted on the tarsal receptors and the smallest effect when they are painted on the receptors of the antennae and the labium.*

The experiments provided some understanding of the mode of action of insect repellents. They suggest that repellents interfere with normal behavior perhaps by blocking the olfactory receptors mediating attraction to food and the contact chemoreceptors invoking feeding on blood and those used in the selection of oviposition sites. The experiments show that mechanoreceptors effecting orientation to gravity and air flow and visual receptors effecting orientation to black stripes are also interfered with by repellents. There is also some evidence that repellents block the thermoreceptors which may mediate piercing for feeding on blood and perhaps auditory organs involved in mating. The only receptors which the repellents do not appear to interfere with seem to be those of the common chemical sense.

INTRODUCTION

The discovery of the transmission of malarial parasites by Ross (1898) and the discovery by Walter Reed and his collaborators that yellow fever was transmitted by *Aedes aegypti* led to the realization of the importance of mosquitoes as carrier of disease. Repellents being a cheap and efficient means of individual protection, many workers studied their effects mainly against the blood feeding behavior of insects. Kalmus & Hocking (1960), however, studied some other aspects of behavior as well. I have studied the behavior of *Aedes aegypti* in the presence of repellents not only in relation to blood feeding but also in relation to sugar feeding, mating, oviposition, geotaxis, wind direction and speeds, and visual responses to black stripes. The repellents used were: dimethyl phthalate, indalone, diethyl toluamide, and Rutger's 612. The first two are esters, the third an amide and the last named an alcohol. These are compounds of low volatility and moderate molecular weight. They are insoluble or only very slightly soluble in water but are miscible with alcohol and ether. Their physical and chemical properties are listed in table 1.

TABLE 1 - Chemical and physical properties of the repellents used in the study of behavior of *Aedes*.

Common Name	Chemical Name	Mol. Wt.	Boiling Point	Solubility and Miscibility
Dimethyl phthalate	dimethyl benzene-ortho-dicarboxylate	194.18	285°C	0.43% w/w soluble in water
Indalone	n-butyl mesityl-oxide oxalate	226.26	113°C	Insoluble in water; miscible with alcohol
Diethyl toluamide	N, N-diethyl m-toluamide	191	111°C at 1mm	Insoluble in water; miscible with alcohol
Rutger's 612	2-ethyl, 1, 3-hexanediol	146.22	244°C	Slightly soluble in water; miscible with alcohol

Blood feeding behavior was studied by applying the repellents in various ways in the environment and on different chemosensory fields of female *Aedes aegypti*. Some general observations were also made on the blood feeding behavior of *Aedes* spp. mosquitoes in the field and *Aedes aegypti* in the laboratory. A preliminary test was made of the effect of washing chemosensory areas with lipoid solvents on blood feeding by *Aedes aegypti*.

REVIEW

Sense Organs of *Aedes aegypti* L.

An *Aedes aegypti* female is attracted to its host in part through the chemoreceptors located on head appendages, mainly the antennae. Bishop and Gilchrist (1946) showed that in *Aedes aegypti* eyes are not essential for feeding on blood. Roth (1951, p. 60) also reported that eyes are not necessary in locating the host in a small cage.

DeLong (1946) considered the antennae and the palps as the chief organs for locating the host and stimulating probing. According to him the antennae may perform both functions but the palps can receive stimuli only when the insect is directly on the skin. Roth (1951) considered that the antennae function as directional thermoreceptors and probably chemoreceptors as well. Roth (1951) also reported temperature receptors on the palps of *A. aegypti*. Dethier (1952) considered that different receptor fields function at different levels of sensitivity. The antennae according to him are the most sensitive and the various mouthparts less so. Rahm (1958) showed by antennal amputation that these organs are essential for host finding and attraction from a distance. He also reported that antenna-less mosquitoes can probe and suck if the palps remain intact.

Antennae

The antennae in the male and female consist of a basal ring-like scape, a globular pedicel, and a long flagellum of thirteen articles. The pedicel in both sexes contains Johnston's organ, which is more developed in the male.

Roth and Willis (1952) reported that many thin walled trichoid sensilla are present on each of the thirteen flagellar articles of the female *A. aegypti* and on the two terminal flagellar articles of the male. They concluded on experimental evidence that they serve as hygroreceptors.

Christophers (1960, p. 663) described the trichoid sensilla as "...40-50 μ in length, thin walled and without articulated base, arising from thin membrane over a pore canal surrounded distally by a semicircular ridge in the article."

Steward and Atwood (1963) identified five structural types of sensilla on the antenna of the female *A. aegypti*. Three of these types they found thin walled and classified them as A1, A2 and A3. According to them a typical A1 sensillum is 0.06 mm long, curved and tapering to a sharp point. Type A2 is shorter, 0.04 mm long and with a blunt tip. Both are about the same diameter. The innervation of the two types is essentially the same. Steward and Atwood described type A3 as a short, curved, thin-walled peg organ which is innervated by a group of nerve cells. Sensilla of type A1 and A2 are more numerous on the distal articles while sensilla of type A3 are found to be located chiefly on the proximal articles of the antennal flagellum. They concluded from experimental evidence that type A1 and perhaps A3 play a major role in mediating attraction while type A2 are responsible for mediating repulsion.

Slifer and Sekhon (1962) studied the structure of the sense organs in the flagellum of *A. aegypti*. The heavy walled hairs according to them are mechanoreceptors. The thin walled hairs with sharp tips they thought to be chemoreceptors. The thin-walled hairs with blunt tips they supposed to be olfactory in function.

Palpi

Roth and Willis (1952) described the palps of female *Aedes aegypti* as abundantly supplied with thin-walled club-shaped sensilla on the terminal segment. Pointed trichoid sensilla are also present. There is also a central short sclerotized peg at the tip of the palp.

Labium

Frings and Hamrum (1950) noted four kinds of hairs on both sexes of *A. aegypti*. Of these, hairs about 40 μ long and lying at the tip of the labella are considered to be tactile in function while curved hairs about 20 μ in length at the tip and on the ventral surface are believed to be chemoreceptors.

Tarsi

On the tarsi of the fore and mid legs of *A. aegypti* are many slightly curved hairs probably tactile in function (Frings and Hamrum, 1950). Wallis (1954) found that in *A. aegypti* all tarsal segments were provided with thin-walled curved spines. Slifer (1962) described the hairs on the tarsi as approximately 100 in number in the female. These hairs stain at the tip when dye is applied to the external surface of the insect. She concluded: "Little doubt now remains that the hairs with stainable tips are the tarsal gustatory receptors of the mosquito."

Mode of Action of Olfactory Receptors

Several theories have been advanced to explain the mode of action of olfactory receptors. Jones and Jones (1953) reviewed the modern theories on olfaction and classified them as; mechanical, chemical, steric, radiation and vibration theories.

Davies (1962) proposed that the mechanism of olfaction is the penetration and dislocation of a small region of the wall of an olfactory nerve cell. This dislocation allows the potassium and sodium ions to move across the membrane, so initiating the nerve impulse.

Amoore (1963), and Amoore, Johnston and Rubin (1964) favor the stereochemical theory of olfaction. According to them the odor of a chemical is determined by the structure of the molecule, in particular by its size and shape. If a chemical is volatile, and its molecules have the appropriate configurations to fit closely into the receptor site, then a nerve impulse will be initiated, possibly through a mechanism involving disorientation and hence depolarization of the receptor cell membrane.

Factors Attracting Mosquitoes to the Host

The mode of action of repellents cannot be fully studied without an understanding of the factors that attract the insect to the host. Contradictory views can be found in the literature on this point; all workers accept temperature and humidity, as attractant factors; others consider factors like carbon dioxide and host odor, or only carbon dioxide to be also important in attracting the mosquito to its host.

Howlett (1910) believed temperature to be the chief attractant and said that the smell of sweat or of blood was not attractive. Reuter (1936) showed that moisture was distinctly attractive to *A. aegypti*. Van Thiel (1937) assigned the role of attraction chiefly to the physical factors of temperature and humidity and the chemical factor, carbon dioxide. Later Van Thiel (1953) considered that the scent of the host plays an important part in the orientation of the mosquito toward it.

DeLong, Davidson, Peffly and Venard (1945) found moistened warm air more attractive to *A. aegypti* than warm air. Most of their tests were conducted with olfactometers or inanimate objects. Brown (1958) recognized six factors which guide female mosquitoes to their animal hosts, three of these being air-borne (water vapor, carbon dioxide, and convective heat) and three visual (movement, contour, and reflectivity).

Kellogg and Wright (1957) and Wright (1962) considered moisture and carbon dioxide to be the main attractant factors. Christophers (1960, p. 535) remarked: "The evidence that smell is an important stimulus in the attraction of *A. aegypti* to feed is not very strong."

On the other hand, many have said that body odor plays an important role in the attraction of mosquitoes. Goeldi (1905) reported perspiration to be the agent attracting mosquitoes to man. Haddow (1942) reported that an unwashed African child attracts more *Anopheles* spp. than a clean child. Willis (1947) reported that females of *A. aegypti* and *Anopheles quadrimaculatus* Say were attracted by the odor of the human arm. He also found CO_2 in concentrations of 1, 10, or 50 per cent in the air not attractive to females of *A. aegypti* or *Anopheles quadrimaculatus* when tested in an olfactometer. Bates (1949) thought smell to be the primary stimulus in guiding the mosquito in its search for food. Rahm (1956) reported that CO_2 emitted by the skin did not determine attractiveness and remarked (1957) that human odor and sweat may play a part in the attraction of mosquitoes to the human hand. Again in 1957 he reported that perspiration did not seem to attract mosquitoes but the odors given out by the host did. Rahm (1958) further remarked that the olfactory substances of man were found to be alone responsible for greater activity of female *A. aegypti*. Dethier (1957) wrote: "Host finding and discrimination, trail following, orientation to odors by flying insects and courtship are shown to depend largely on the chemical stimuli."

EXPERIMENTAL - BEHAVIOUR

Blood Feeding in Relation to Repellents

Christophers (1960, p. 486) remarked on blood feeding by *A. aegypti* in the following words: "Another striking feature of feeding is that the insect once it has begun to suck blood, appears to become oblivious to all danger and considerable physical force is required to make it give up its hold." This feature is referred to by Gordon and Lumsden (1939) who wrote that they were only able to get *A. aegypti* to feed on the frog's foot by employing mosquitoes which had been allowed to start feeding on the human arm. When nearing repletion, however, the insect usually leaves readily if disturbed.

Kalmus and Hocking (1960) observed the effect of painting repellent with a fine camel hair brush on the backs of feeding mosquitoes. A lead was taken from this study and more observations were made on the effect of repellents on other species of *Aedes* in the field and *Aedes aegypti* in the laboratory.

Observations on Aedes *spp. in the Field*

For studies on the species of *Aedes* in the field a thicket of poplar trees was selected. The four repellents, dimethylphthalate, ethyl hexanediol, indalone, and N-N-diethylmetatoluamide were used. The mosquitoes reacted to all four repellents in the same way. The species of *Aedes* studied were *A. punctor* Kirby, *A. cataphylla* Dyar, and *A. intrudens* Dyar.

The time to take a complete blood meal, from the insertion of the proboscis to its retraction after complete engorgement ranged from two to four minutes. (Mean = 2 min 31 sec with standard deviation 41 sec). It was observed that the mosquitoes could be very easily disturbed in the early stages of their blood meal. If a clean brush were brought near them soon after the insertion of the proboscis, they could be seen retracting it. If a repellent or olive oil were placed near the antennae or painted on the mesonotum, the mosquitoes invariably flew away. As reported by Kalmus and Hocking (1960, p. 7) "A contact between repellent chemicals as liquids and substantial areas of the proboscis, tarsi and tibiae, mesonotum or the wings leads to the interruption of biting, and in mosquitoes not engaged in biting to the retraction of the touched limb or limbs or to take off." But the behavior of mosquitoes was found quite different in relation to repellents and other stimuli if they had been feeding for a minute or more, i. e. roughly in the middle of their meal; e. g.:

(i) The mesonotum was rubbed with a dry brush, painted with repellents or olive oil until the whole mesonotum was covered with liquid, but the mosquito never flew away, instead it completed its blood meal, continuing to feed for another 45 seconds to one minute.

(ii) The antennae were painted with repellents, were in fact soaked in repellent, but the mosquitoes continued to feed.

(iii) A drop of repellent was made to flow near the tarsi, there was no reaction until it made contact with them. As soon as contact was made the tarsus was lifted. The same reaction was observed with olive oil. However, the mosquitoes continued to feed even when the tarsi of all the six legs were lifted. The mosquito then came to rest on its abdomen. When the repellent was presented on a brush near the lifted tarsi, they sometimes rested the tarsi on the repellent soaked brush, without showing any other abnormal behavior, and continued to feed.

(iv) Similar behavior was observed in mosquitoes feeding on the foot through socks. Mosquitoes coming to feed landed only on clean areas of the sock and avoided areas where repellent had been placed. However, mosquitoes which had been feeding through the sock for some time were not affected if a repellent was placed on the sock underneath them, and they continued to feed to completion although they lifted the abdomen.

(v) Chloroform or ether was brought near the abdomen of a feeding mosquito. It always flew away, even when it had been feeding for a minute or more.

(vi) A hot spatula was brought near the mosquito (about 1 mm). The spatula was heated for two minutes in a flame of a spirit lamp. Eighty per cent of the mosquitoes took off in 5 to 10 seconds. When the spatula heated for the same time was kept at the same distance from the mercury bulb of a Fahrenheit thermometer, the thermometer registered a rise of 4-6 degrees.

(vii) Repellent was painted on the wing of a feeding mosquito. The mosquito always flew away but when the wing was rubbed with a dry brush or painted with olive oil it continued to feed.

(viii) Physical injury was inflicted on the mosquito to the extent that all the six legs were clipped off at the femoro - tibial joint, but it continued to feed and did not fly away.

The observations were made at a temperature of 65°F and R. H. of 57%.

Observations on Aedes aegypti

In the laboratory the same behavior was studied in *Aedes aegypti*. A one cubic foot cage made of steel wire and covered with nylon net was fitted with a sleeve on each of two adjacent walls, i. e. at right angles to one another. Mosquitoes were allowed to feed on a hand inserted through one sleeve while the other hand was introduced through the other sleeve to apply the repellent.

As observed in the other species of *Aedes*, *Aedes aegypti* could also be easily disturbed in the initial stages of blood feeding, but after one minute of feeding they could not be disturbed so easily:

(i) When the mesonotum was rubbed with a dry brush or painted with olive oil or any of the four repellents under study.

(ii) When their wings were painted with repellents. This was contrary to the behavior observed in the field species which invariably flew away whenever repellents were painted on the wings.

(iii) They continued to feed even when they were made to rest their tarsi on the repellent soaked brush.

(iv) Being small in size, it was not possible to paint their antennae with repellent while they were feeding, but when a drop of repellent was placed very close to the proboscis they continued to feed.

(v) Almost every mosquito continued to feed when the tarsi of its hind legs were clipped off, but some flew away when the tarsi of their other legs were clipped.

(vi) When a heated spatula was brought near them they always flew away even when the spatula was as far as 1-2 cm away. It had to be brought much nearer to mosquitoes in the field to elicit this response. When the spatula heated for the same time was kept at the same distance from the mercury bulb of a Fahrenheit thermometer this registered a rise of 1.5 to 2 degrees.

Experiments were conducted by applying the repellent on different chemosensory fields of female *A. aegypti* and observing the behavior and recording the number feeding on an untreated human arm. As the repellent was not applied on the skin, there was no interaction between the skin and the repellent or the chemical stimuli emanating from the skin and the repellent on the surface of the skin. The experiments provided some understanding of the site of action of different repellents as well as providing a quantitative basis for comparing the repellents with each other. The experiments also provided a quantitative basis for evaluating the function and efficiency with which the different chemosensory fields play their role in the act of feeding as well as some grounds for accepting the role of smell in attracting mosquitoes to feed and the function of the repellent when applied on the skin in offsetting this role.

10-12 female mosquitoes, 7-8 days old, previously fed on raisins and sugar solution only, in a sucking tube and then chilling them for 1.5 min at 15°F, in order to immobilize them. Their probosicides, either one or both antennae, or all the tarsi, were then painted with repellents with a fine brush in separate sets of experiments. This operation was performed over a cold petri dish covered with a filter paper and placed under a binocular microscope. A radius was drawn in ink on the filter paper and mosquitoes were treated one by one, starting on one side of the radius until all of them were treated. They were then sucked back into the sucking tube and released in a paper lined petri dish to revive in a one cubic foot cage of steel wire covered with nylon net. The mosquitoes recovered from the chill in 2-3 minutes. The behavior and the number that fed on blood on introducing the arm into the cage through a sleeve were noted, firstly ten minutes after the treatment and then at greater intervals from the treatment until the number fed in a given time approached the number fed in controls. Two controls were run with each set of experiments, one a plain control when the receptor field that was intended to be treated was rubbed with a dry brush only, and another when it was painted with olive oil. The palps could not be treated separately without running some repellent on the proboscis and the antennae, because of their close proximity to these structures.

Results - The figures given in table 2 give the cumulative mean percentages of mosquitoes feeding on blood after different chemoreceptor sites were painted with repellents. The standard error of the mean was used to find statistical significance between the means.

The results show that Rutger's 612, diethyl toluamide, and indalone reduce the number of mosquitoes feeding on blood more than dimethyl phthalate after the first ten minutes when the proboscis was painted, and the effect lasted longer. Indalone remained significantly more effective as compared to Rutger's 612 and diethyl toluamide after two hours when painted on the proboscis.

When painted on both the antennae, diethyl toluamide, Rutger's 612 and indalone again reduced the number of mosquitoes feeding more than dimethyl phthalate. The effect of dimethyl phthalate was found to have been lost within one hour but the effect of the other three repellents lasted more than six hours.

When painted on one antenna, the same significant differences were found between the repellents as when both the antennae were painted, i.e., diethyl toluamide, Rutger's 612 and indalone were significantly more effective than dimethyl phthalate.

The results obtained on painting all the tarsi with repellents were, however, different. Dimethyl phthalate was found to reduce feeding more effectively when painted on tarsi than when painted on both the antennae or on the proboscis, and to maintain this effect at least as long as the other three materials.

There is evidence that many repellents work by way of specialized chemoreceptors (Weismann and Lotmar, 1949; Dethier and Yost, 1952; Peters, 1956; Dethier, 1956 a). Peters (1956) reported that *Calliphora erythrocephala* could detect dimethyl benzamide with the tarsal

TABLE 2 - Cumulative percentages of *A. aegypti* females that fed after different chemoreceptor areas were painted with repellents. Means of four replicates ± standard errors.

Chemoreceptor area painted	Observation time	Control	Olive Oil	D. M. P.	D. E. T.	Rutger's 612	Indalone
Proboscis	10 min	82 ± 3.1	73 ± 2.5	33 ± 3.6	12 ± 3.9	22 ± 5.5	10 ± 3.5
	1 hr	--	--	72 ± 4.4	51 ± 8.7	52 ± 3.7	47 ± 3.2
	2 hr	--	--	75 ± 4.7	72 ± 2.9	76 ± 5.7	63 ± 2.6
	3 hr	--	--	--	--	--	71 ± 1.6
Both Antennae	10 min	76 ± 2.3	71 ± 4.1	33 ± 2.3	3 ± 3	0	3 ± 3
	1 hr	--	76 ± 2.5	81 ± 4.2	6 ± 3.2	5 ± 2.8	8 ± 4.8
	2 hr	--	--	83 ± 2.3	11 ± 4.1	11 ± 4.1	19 ± 5
	6 hr	--	--	--	32 ± 3.1	33 ± 4.5	30 ± 3.1
One Antenna	10 min	85 ± 2.9	80 ± 4	61 ± 3.3	5 ± 2.7	25 ± 5.9	19 ± 3.3
	1 hr	--	--	80 ± 4	38 ± 10	49 ± 4.3	56 ± 2.4
	2 hr	--	--	--	75 ± 2.3	69 ± 3.2	72 ± 4.5
Tarsi	10 min	80 ± 4	73 ± 2.5	27 ± 2.7	14 ± 5.2	27 ± 2.9	30 ± 3.8
	1 hr	83 ± 2.5	78 ± 2.5	50 ± 8.4	61 ± 2.3	71 ± 3.9	74 ± 3.5
	2 hr	--	--	68 ± 1.5	76 ± 2.5	76 ± 2.2	79 ± 1.5

receptors only, while other materials like indalone and dimethyl carbate could be detected with the tarsal receptors, labella, and antennae.

The significant difference in the number of mosquitoes landing on the hand after treatment of chemoreceptors on different head appendages and on the tarsi can be explained on the basis of the population of chemoreceptors getting such treatment. As most of the chemoreceptors are situated on the antennae, their treatment with repellents would inhibit the landing of mosquitoes on the hand more than the treatment of other head appendages. The ineffectiveness of the painting of one antennae only in keeping the mosquitoes from a blood meal for two hours can be explained by the same argument, i.e., a large population of chemoreceptors remained functioning effectively when only one antenna was painted. The painting of any one of these chemoreceptor sites with repellent must be affecting the mosquito in two ways, affecting the chemoreceptors of the chemosensory area painted in liquid form and also affecting the adjacent chemosensory sensilla in vapor form. The greater the area painted, the greater the number of sensilla affected, resulting in inhibition of feeding for a longer period.

Painting Repellent on Mosquito Antennae and Host Skin

By the procedure described above one antenna of each of about 10 *A. aegypti* females was painted with diethyl toluamide. An arm also treated with diethyl toluamide was then introduced into the cage and the behavior of the mosquitoes was studied. A little more flight activity and some searching on the wing was observed in these mosquitoes as compared to those in the control where no repellent was applied on the mosquitoes themselves but only on the hand. A similar behavior was observed in experiments with the other three repellents as well. In controls, mosquitoes were seen mostly sitting on the walls of the cage. There was little or no flight activity.

When both the antennae of mosquitoes were treated with diethyl toluamide, indalone or Rutger's 612, and the same repellent was applied on the hand introduced into the cage, the mosquitoes could be seen searching on the wing. Many landed on the repellent coated surface of the hand, walked about and even probed but did not take a blood meal. The behavior was observed for ten minutes every hour for four hours but no mosquito bit. In similar experiments with dimethyl phthalate, however, no landings on the hand were observed though the mosquitoes came quite close to it and sometimes even touched the skin.

Sugar Feeding

The principal food of female *Aedes aegypti* is blood from a human host though they can exist for long periods on food other than blood. Male *Aedes aegypti* do not take blood at all but feed entirely on sugary materials. Goeldi (1905) kept females alive for 31 to 102 days on honey alone. Macfie (1915) observed that the females feed on honey for the first couple of days but the males feed only on honey at anytime. Gordon (1922 b) observed both males and females of *Aedes aegypti* sucking nectar from flowers. Many observers have noted that sugary fluids, raisins, bananas, and other fruits are sucked by both sexes.

Many workers have devoted much time to studies of the effect of repellents on blood feeding of mosquitoes but their effect on sugar feeding has not attracted much attention. Evans (1961) has studied the effects by the blowfly *Phormia regina* Meigen. Experiments were conducted to study the effect of repellents on the feeding of *Aedes aegypti* on raisins.

Kalmus and Hocking (1960) conducted some tests on blood feeding in relation to repellents with *Aedes aegypti* by keeping a 10 cm length of 3mm outside diameter glass tubing which was clamped in a vertical position so that the lower end was about 1 cm above the middle of a 6 cm bare circle on the back of a gloved hand. A few drops of repellent were placed in the lower end of the tube. In this way a circle of skin about 1.5 cm diameter was kept free of bites. A lead was taken from this experiment in exploring the effect of repellents on the feeding of *Aedes aegypti* on raisins.

Experiment 1

About 100 male and 100 female mosquitoes were taken in a cubic foot cage of steel wire covered with nylon net. The age of the mosquitoes was 2-4 days and they were not fed anything for six hours prior to experiments. Ten raisins were fixed with 1 cm clear space between each on a horizontal steel wire hanging 4 inches below the top of the cage. The wire was hung by bending its ends and hooking them on top of the side walls of the cage. A 2 cm wide strip of paper was fixed above the raisins, running parallel to them at a distance of 1.5 cm. Half of this paper strip (covering 5 raisins) was painted with repellent and the other half (covering the other 5 raisins) was kept as a control. Observations were made on the number of mosquitoes settling on either side at intervals of 5 minutes. After each observation the cage was shaken and another observation recorded after five minutes. In this way five replicates were taken for each repellent. Separate batches of mosquitoes were taken in separate cages for experiments with different repellents.

The observations are recorded in table 3. The vapor of repellents significantly reduced the number of mosquitoes feeding on raisins. The standard error of the mean was used as statistical test for significance.

Experiment 2

Ten raisins were fixed on the wire lying as close to each other as possible without touching. Five alternate raisins were then painted with repellent leaving the other five as controls. The numbers of mosquitoes that settled on the treated and untreated raisins are recorded in table 4, column 1 to 3. The figures are means of 5 replicates. Observations were recorded every five minutes as in the previous experiment. The total number of mosquitoes in the cage for each experiment was 200.

In these experiments mosquitoes were seen coming close to the raisins to land but they usually flew away without landing. No significant difference was found between the number of mosquitoes settling on treated and untreated raisins in the control with olive oil. The results show that the repellent on the treated raisins kept the mosquitoes away from the untreated raisins as well. Kalmus and Hocking (1960, p. 23) obtained bites up to almost a mosquito half-width (about 2.3 mm) from a repellent painted circle on the back of the hand. In these experiments the mean width of untreated raisin separating the two treated ones with repellent was 10 ± 0.3 mm. This greater distance was perhaps due to the factors of heat, CO_2 and probably skin odor, which were missing as attractant factors in the raisins.

TABLE 3 - Numbers of *A. aegypti* settling on raisins separated by 1 cm, under the plain and repellent coated halves of a paper strip. Means of five replicates ± standard errors.

Paper strip half	Olive oil	D. M. P.	D. E. T.	Rutger's 612	Indalone
Painted with chemical	18 ± 1.9	5 ± 0.5	3 ± 0.7	3 ± 0.1	2 ± 0.8
Plain	19 ± 4	18 ± 1.9	12 ± 1.3	13 ± 1.4	10 ± 2.2

Experiment 3

Raisins were kept 1 cm clear apart from each other and alternate raisins were painted with repellent. Other factors were the same as in the previous experiments.

The mean numbers of mosquitoes that landed on the treated and untreated raisins are given in table 4, columns 4 and 5. The numbers of mosquitoes feeding or settling on the untreated raisins were still very low and no significant difference was found in the number of mosquitoes feeding on untreated raisins in this experiment as compared to the number of mosquitoes feeding on untreated raisins in the previous experiment.

Experiment 4

Only 5 raisins were taken and were placed 1 cm clear apart and the portion of wire between them was painted with repellent. Since the raisins were not painted with repellent in this experiment their number was reduced to five so that the number of mosquitoes landing on them could be compared with the number of mosquitoes landing on the untreated raisins in previous experiments.

The results are given in table 4, column 6. The comparison of results in table 4 shows that significantly more mosquitoes settled on raisins in this experiment than in experiments where treated and untreated raisins were placed close to each other. This is perhaps due to the small surface area of wire between the raisins as compared to the much greater area of the raisins in the previous experiments. This would result in a much slower production of repellent vapor.

TABLE 4 - Numbers of *A. aegypti* settling on raisins in the presence of repellents. Means of five replicates ± standard errors.

	Raisins close together, alternate raisins painted with repellent		Raisins 1 cm apart, alternate raisins painted with repellent		Raisins 1cm apart & the wire in between painted with repellent
Chemical	Untreated raisins	Treated raisins	Untreated raisins	Treated raisins	Untreated raisins
Olive oil	16 ± 2	14 ± 4	10 ± 0.7	11 ± 1.4	10 ± 1
D.M.P.	1	0	2 ± 0.5	0	2 ± 1
Rutger's 612	1	0	2 ± 0.8	0	3 ± 0.5
Indalone	0	0	0	0	3 ± 1
D.E.T.	0	0	2 ± 0.5	0	5 ± 0.5

Mating

In *Aedes aegypti* "The stimulus which induces the male to copulate is the sound produced by the female during flight." ".. odor plays no part in the sexual behavior of *aegypti* ..." (Roth, 1948, pp. 284, 282). Roth also observed that in *Aedes aegypti* the male is the aggressor and is attracted by the female in flight and that the female is passive and does not show any mating behavior similar to that of the male. "...never in our observations was a male seen to initiate copulation with a resting female" (Roth, 1948, p. 276). Banks (1908, p. 246) on the contrary stated that specimens of *aegypti* confined in small jars"...have been seen to copulate while the female hangs from the gauze covering the vessel, the male always approaching her from the ventral surface." Christophers (1960, p. 502) observed that copulation takes place quite commonly with the female at rest.

During the course of this work it was observed that a female *Aedes aegypti* is not entirely passive and that copulation does take place when a female is at rest. It was observed that when a flying male came close to a sitting female, the female would take flight and the male would grasp her for copulation. Many times females were seen taking flight spontaneously and males were seen getting hold of them in mid-air. The males were also observed coming to land sideways with a female, then trying to take a ventral position and many a time they succeeded. At other times because of his efforts to gain a ventral position to the resting female the male roused the female to fly and copulation took place on the wing or the two could be seen falling to the floor copulating. But mostly copulation took place with a female in flight.

Roth (1948) also observed that the male would copulate repeatedly with the same or different females. After repeated matings, females become more and more reluctant to fly and would resist the attempts of the males to copulate. Richards (1927) suggested that repeated copulations exhaust the individuals. Shannon and Putnam (1934) in their laboratory study of *A. aegypti* observed that the average pupal period of females was 14 hours longer than that of males. Roth (1948, p. 308) observed that by the time the female begins to fly and becomes 'attractive' the male's antennae have reached a state where the sound stimulus can be perceived and his genitalia have rotated sufficiently so that copulation can be successful (usually about 15 to 24 hours after emergence). In view of these observations it was necessary in this work to separate the sexes before they started mating and to keep the observation time reasonably short. To forestall fatigue in the females due to repeated copulations, the males were separated from the females 14 hours after emergence.

Ten females 2-4 days old and 10 males 5-6 days old were used for each experiment. The females were chilled in a sucking tube for 1.5 minutes at 15°F and then all their tarsi were painted with repellent with a fine brush while on a cold petri dish under a binocular microscope. Since *Aedes aegypti* mate venter to venter and the female does not clasp the male to her, her legs remaining out-stretched and serving as structures to which the male clings (Roth, 1948, pp. 270, 301), it was decided to paint the tarsi of the female mosquito with repellent. After the tarsi were painted the females were released in a one cubic foot cage and allowed to recover from chill. They recovered in 3-4 minutes. Ten minutes after the treatment 10 males were released in the cage. After application of the repellent on the tarsi of the female *Aedes aegypti* few flew spontaneously. Most females sat quietly on the walls of the cage. Males hardly ever succeeded in persuading the female at rest to copulate. It was also observed, though no quantitative basis could be laid down for this, that the efforts of the male to copulate with the resting female, as well as with the female in flight, were less persistent and quite often they were seen releasing the female soon after coming in contact. The cage was therefore shaken every minute to make the females fly and the number of matings in a period of 30 minutes was recorded. Each experiment was performed with a new batch of mosquitoes.

The results are recorded in table 5. The standard error of the mean was used as a test of significance. The highly significant reduction in the number of matings in *A. aegypti* in association with repellents can be explained as a result of two factors: 1) a decrease in the flying activity of the females and 2) less persistent efforts by males and premature release of the female.

Though the cage was shaken every minute in experiments with repellents as well as in the control it was observed that the females in the controls continued to fly for a much longer time after shaking than in experiments with repellents. With repellents, most of the time the females could be seen coming to rest on the wall immediately after shaking the cage, and many a time on shaking they would fly only from one wall of the cage to another. There was also a lack of spontaneous flight activity on the part of the females.

TABLE 5 - Numbers of matings in a 30 minute period in a population of 10 male and 10 female *Aedes aegypti* with repellents applied to the tarsi of the females. Means of four replicates ± standard errors.

Control	Olive oil	D. M. P.	Indalone	Rutger's 612	D. E. T.
65 ± 2	65 ± 1	33 ± 2	30 ± 3	33 ± 3	33 ± 3

Oviposition

Wallis (1954) in his studies on the oviposition activity of mosquitoes, including *A. aegypti*, found that the female could detect an objectionable amount of salt even when the movements of the abdomen were restricted. Likewise surgical removal of the palpi, proboscis, and antennae from the head did not result in loss of sensitivity. Surgical removal or wax coating of various combinations of legs and leg articles resulted in the demonstration that sensitivity was localized in the tarsal articles of all the species of mosquitoes studied by him. His investigations also showed that the sensitivity was present in all the tarsal articles of *Aedes aegypti*. The thin walled chemoreceptors of the tarsi enabled the mosquitoes to detect differences in saline concentrations as slight as 0.02 M.

Browne (1960) studied the role of olfaction in the stimulation of oviposition in the blowfly *Phormia regina* Meigen. He found that the odor of a liquid medium containing powdered milk and yeast stimulated the blowfly to oviposit. He also provided evidence for olfactory perception by the ovipositor of the blowfly.

In this study oviposition in *Aedes aegypti* was observed by associating potential oviposition sites with repellent vapors as well as by applying repellents on the tarsal chemoreceptors.

Experiment 1

Five, 7-8 day old blood fed females in a one cubic foot cage were taken for each experiment. The cage was provided with a rectangular platform, 7" x 4" made of a steel wire frame (diameter of wire 2.5 mm). The platform was covered with nylon net on one side and with two paper towel strips pasted on the other except in the center where a gap of 1 cm was left in between the strips, see figure 1.

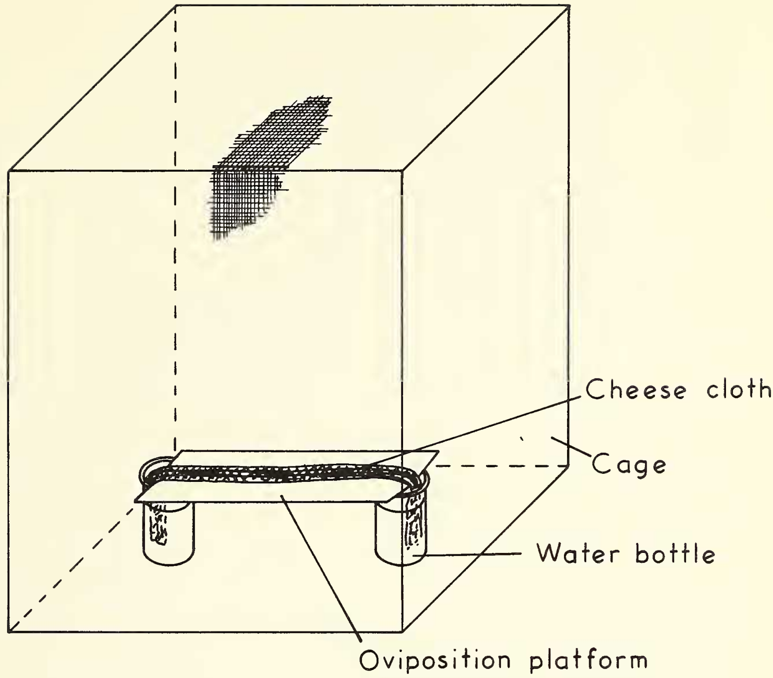
The platform was placed in the cage, nylon net side upwards, the ends resting on two glass bottles filled with water. On the nylon net was spread a piece of cheese cloth, the two ends of which remained dipped in the water in the glass bottles. The cheese cloth was kept wet by capillary action by the water in the two bottles. One of the two paper strips was painted with repellent while the other was left untreated. Thus an oviposition platform for the mosquitoes was provided, one half of which had repellent vapor coming from underneath through the nylon screen, while the other half served as control. The nylon net underneath the cheese cloth served as a support for it and did not allow it to come in contact with the repellent on the paper strip below but allowed the repellent vapors to pass through. Most of the eggs were found to be laid on the cheese cloth but some were laid on the paper strip. They were counted separately 72 hours after the blood meal, and the results are recorded in table 6. Four experiments were run with each repellent.

The behavior of *Aedes aegypti* during egg laying is described in detail by Wallis (1954). During the experiments it was observed that a female mosquito could sample the oviposition sites while on the wing and would land on the control half rather than on the repellent treated half of the oviposition platform. At other times when she landed on the repellent half she walked for a few seconds and then flew away and landed on the control side. This behavior demonstrates the function of olfactory receptors in the selection of an oviposition site when repellent vapors are associated with it. The complete absence of egg laying on the repellent coated as well as olive oil coated paper towels on the lower side of the platform seems to be the result of tarsal chemoreceptors which select the suitability of the egg laying medium on contact. The significantly small numbers of eggs laid on cheese cloth on the repellent side as compared to the number of eggs laid on the control side show that *Aedes aegypti* rejects oviposition sites when these are associated with repellent vapor.

Experiment 2

Experiments were also conducted by painting the tarsi with repellent by the same technique as described in previous experiments and recording the number of eggs laid in 24 hours. Christophers (1960, p. 507) records that egg laying in *Aedes aegypti* usually begins on the afternoon of the third day from blood feeding, counting the day of feed as zero. Female mosquitoes 6-7 days old were fed on blood and left in a cage with raisins for three days. On the fourth day their tarsi were painted with repellent and the mosquitoes were placed singly in separate vials with water soaked cotton wool in the bottom and a nylon net cap on the top on which was placed a raisin. Eggs laid in a 24 hour period were then counted. Four replicates were run for each experiment. The mean numbers of eggs laid are recorded in table 7.

The difference in the number of eggs laid in the control and those laid by repellent treated mosquitoes is not significant, using standard error of the mean as a test of significance. This is perhaps a.



b.

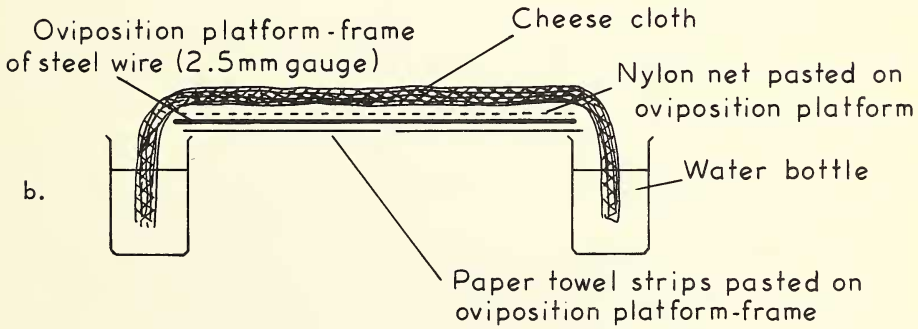


Figure 1. Diagrams showing: (a) arrangement of the oviposition platform in the cage, and (b) a vertical section of the oviposition platform.

due to the fact that the mosquitoes had no opportunity to select a site for oviposition as they were confined in small vials.

TABLE 6 - Numbers of eggs laid by *A. aegypti* females in the presence of repellents. Means of four replicates ± standard errors.

Chemical	Eggs laid on control side: On cheese cloth	Eggs laid on control side: On paper towel	Eggs laid on repellent side: On cheese cloth	Eggs laid on repellent side: On paper towel
Rutger's 612	128 ± 18.6	52 ± 4.2	0	0
D. E. T.	160 ± 20.5	47 ± 2.2	2 ± 1.7	0
Indalone	156 ± 18.4	19 ± 1.5	0	0
D. M. P.	208 ± 13	22 ± 1.7	5 ± 1.1	0
Olive oil	87 ± 6	12 ± 1.1	68 ± 7.2	0

Experiment 3

Experiments were also conducted to determine whether antennectomized mosquitoes would discriminate between the control and the repellent sides of the oviposition site. Twenty female mosquitoes which had been fed on blood previously were operated upon for each experiment on a cold petri dish under a binocular microscope after first chilling them for 1.5 minutes at 14°F. Ten to 12 flagellar segments of the antennae were excised and the mosquitoes then released in the cage with the oviposition platform shown in figure 1.

Though sometimes mosquitoes could be seen sitting on the control side of the egg laying platform, no eggs were laid in any of the experiments over a week's time except in the experiment with diethyl toluamide where there were 4 eggs on the control side. A high mortality (70-75%) was also observed in mosquitoes during this period. The almost complete absence of oviposition by antennectomized mosquitoes may be due to lack of orientation of mosquitoes to the water soaked cheese cloth on account of the great reduction in the number of hygroreceptors as a results of excision and consequently a great increase in the threshold of moisture perception. The high mortality rate can also be assigned to the same factor, i.e., lack of orientation to the water soaked cheese cloth and hence dehydration. Mosquitoes were seldom seen sitting on the wet cheese cloth. Most of the time they were found sitting on the walls of the cage with very little flight activity. The very low activity in antennectomized mosquitoes confirms the findings of Bar-Zeev (1960) who found only 4 per cent of mosquitoes could be activated when antennectomized as compared to 60.1 per cent when intact.

TABLE 7 - Numbers of eggs laid by single *A. aegypti* females after painting the tarsi with repellents. Means of four replicates ± standard errors.

Control	Olive oil	D. M. P.	Rutger's 612	D. E. T.	Indalone
39 ± 8.8	42 ± 4.8	29 ± 5.2	34 ± 8.6	34 ± 5	33 ± 4.4

Experiment 4

Experiments were also conducted to test oviposition after treating the terminalia of the females with repellent. The female *aegypti* mosquitoes were fed on blood when 7-8 days old, and their terminalia painted with repellent 72 hours after the blood feed by the same technique as described in the previous experiments, and then released in the cage.

All the mosquitoes became too crippled to move about or fly shortly after the painting of the tip of the abdomen and died in a few hours.

Behaviour in Relation to Wind Direction and Speed

Kalmus and Hocking (1960, p. 21) conducted a series of experiments in which target areas were drawn out on the backs of subjects who wore shirts with the backs cut out. They recorded the distribution of bites in relation to a small repellent treated area. To demonstrate the effect of wind direction on the distribution of bites in relation to repellent, experiments were conducted in the laboratory on *Aedes aegypti* using the same technique.

Experiment 1

A circle of 3.5 cm radius was drawn in hard clear nail varnish on the bare chest of a subject. Concentric to this another circle of 6.5 cm radius was drawn. The outer circle was divided into two equal halves by drawing a diameter. A hair drier was used to produce the air current and a variable transformer was included in the circuit to permit adjustment of the speed of the wind. The wind speed was kept at 43 cm/sec and its direction at right angles to the drawn diameter. The source of wind, i.e., the nozzle of the blower was kept 23 cm away from the central circle which was coated with repellent. The blower was kept in such a position as to give a uniform flow of air over the marked area. The repellent used was diethyl toluamide. One hundred 7-8 day old female *Aedes aegypti* mosquitoes were taken in a one cubic foot cage of steel wire with nylon net around it for each experiment. The mosquitoes were fed on sugar solution only before the experiment. The cage was placed on the marked area and the portion of skin outside the marked area was covered with a polyethylene sheet. Mosquitoes soon started biting through the nylon net on the floor of the cage. An observer kept a record of the mosquitoes that settled and flew away, or settled and bit, in the upwind and downwind halves of the circle. The counts were made for 5 minutes in each experiment. Controls were run with the same wind speed without repellent. The number of mosquitoes that settled or bit in the upwind and downwind halves of the circle are given in table 8.

The results show that in the control where repellent was not painted in the central circle, significantly more mosquitoes settled or bit on the downwind side of the circle than on the upwind side. This is in conformity with the observations made by Kalmus and Hocking (1960, p. 4) with field mosquitoes. However, when repellent was painted in the central circle it was observed that the number of mosquitoes settling or biting on the downwind half of the outer circle was significantly lower than the number settling or biting on the upwind half. This was due to the presence of repellent vapor carried by the wind on the downwind half of the outer circle.

TABLE 8 - Numbers of *A. aegypti* females settling or biting in relation to wind direction and D. E. T. on the marked area of skin.

Wind speed	Control*		D. E. T. **	
	Upwind	Downwind	Upwind	Downwind
43 cm/sec	15 ± 2.5	27 ± 1.6	35 ± 2.9	8 ± 1.7

* Means of two counts ± standard error
** Means of three counts ± standard error

Experiment 2

In another set of experiments the effect of different wind speeds was determined on the settling and biting of mosquitoes in relation to repellent. Experiments were conducted in a similar fashion as described under the experiments with different wind directions, except that the portion of the body used was the thigh instead of the chest, which gave the advantage of the subject himself making notes of the number of mosquitoes landing or biting. A control was run with each wind speed and all the controls with different wind speeds were run first in order to avoid contamination of skin area with repellent vapors. After the controls were run, different batches of mosquitoes were then used in experiments with the same wind speeds in relation to repellent painted in the central circle. The repellent used was diethyl toluamide. The portion of skin outside the outer circle was covered with polyethylene sheet and the count of mosquitoes settling or biting in the upwind or downwind half of the circle was recorded for five minutes in each experiment.

The results are shown in table 9. In previous experiments with different wind directions the number of mosquitoes settling or biting in the upwind half of the circle in experiments with repellents was significantly higher than the number of mosquitoes in the downwind half of the circle. The results given in table 9 show that the mosquitoes continue to show the strong tendency of settling more on the upwind side in relation to repellent with different wind speeds.

The maximum wind speed at which mosquitoes were able to settle on a bluff body was reported to be 95 cm/sec and that of settling on the streamlined body to be 55 cm/sec. Kalmus and Hocking (1960, p. 15). In this case the maximum speed of wind at which the mosquitoes settled on the skin was 265 cm/sec which is very high as compared to the wind speed with the bluff or streamlined bodies. This is probably due to the attractant factors of the skin acting on the mosquitoes.

TABLE 9 - The number of *A. aegypti* females settling or biting in the upwind or the downwind half of the circle marked on skin in relation to different wind speeds and diethyl toluamide.

Wind speed	Control		D. E. T.	
	Upwind	Downwind	Upwind	Downwind
0 cm/sec	27	29	23	19
43 cm/sec	13	26	41	12
134 cm/sec	16	31	9	4
190 cm/sec	5	14	3	1
227 cm/sec	4	8	5	0
265 cm/sec	4	6	2	0
314 cm/sec	0	0	0	0

Orientation to Gravity and Centrifugal Force

Experiment 1

To study the orientation of *Aedes aegypti* to gravity in relation to repellents, experiments were conducted in a plastic petri dish of 9 cm diameter. The lid of the petri dish was perforated with 2 mm diameter holes, about 9 holes per sq cm to allow the repellent vapors inside the dish to escape. The floor of the petri dish was lined with a filter paper which was divided into four quadrants designated top, left, bottom, and right.

Twenty female mosquitoes, 7-8 days old were taken, chilled for 1.5 minutes at 14°F and then released in the petri dish. On recovery of mosquitoes from chill the petri dish was turned with a diameter vertical and given five complete turns on the horizontal axis through its center; thereafter the position and the number of mosquitoes was noted in each quadrant after a minute. The experiment was replicated five times without repellent as a control. A band of repellent 1 cm wide was then painted on the outer margin of the top quadrant. Mosquitoes were chilled and placed in the petri dish and allowed to recover. After the mosquitoes had completely recovered, the dish was given five complete rotations as in the control, keeping it vertical and rotating it about its horizontal axis. The experiment was repeated five times with each repellent.

In the control the mosquitoes could be seen walking upwards and most of them collected in the top quadrant. Significantly less mosquitoes remained in other quadrants. Almost all the mosquitoes were seen facing upwards and the root mean square deviation of their body axes from the vertical axis of the petri dish was found to be zero.

With repellent significantly less mosquitoes entered the top quadrant. Most of them remained in the left, right, and bottom quadrants. They were also seen walking at an angle to the repellent or turning away from it. Their angle of turning (i.e., the angles which the longitudinal axes of the bodies formed with the vertical axis of the petri dish) was noted by marking their position in each quadrant on a separate sheet of paper and then measuring the angle and direction of inclination to the vertical.

Table 10 shows the distribution of mosquitoes in the various quadrants of the petri dish in the presence of repellents, and table 11 shows the root mean square of the angle of inclination of the body axes of mosquitoes to the vertical in the presence of repellents in the petri dish.

Results with olive oil were not found to be significantly different from those of the plain control.

The effect of the presence of repellent on the head upwards orientation of the mosquitoes in relation to gravity was highly significant.

Experiment 2

The effect on geotaxis of painting repellent on the mesonotum and the antennae was also observed. Seven to 9 days old female mosquitoes were chilled for 1.5 minutes at 14°F and their mesonota or antennae were painted with repellent. They were then placed in a 9 cm petri dish having holes in the lid and lined with filter paper. After complete recovery of the mosquitoes from chill the dish was held with its central axis horizontal and rotated slowly about this, one rotation in 20 seconds, and the positions of the mosquitoes were noted. Normal female *A. aegypti* show a counter rotation to maintain a head upward under these circumstances (Kalmus and Hocking, 1960, p. 8).

The mosquitoes with their mesonota painted oriented facing upwards by counter rotation but when the antennae were painted with repellent, on placing the dish in a vertical position the mosquitoes could be seen sitting on the vertical surface head upwards cleaning their antennae with the tarsi of the forelegs. When the dish was rotated slowly while they were cleaning their antennae, they did not react until they faced downwards. Then they were found to lose their balance and were seen to place their forelegs on the vertical surface. Some of them turned around, faced upwards and started cleaning the antennae again, but typical counter rotation was absent.

TABLE 10 - Numbers of *A. aegypti* females found in different quadrants in relation to repellents. Means of five replicates ± standard errors.

Chemical	Quadrants Top	Left	Bottom	Right
Control	15 ± 1	2 ± 0.5	1 ± 0.4	2 ± 1
Olive oil	13 ± 1.6	3 ± 0.8	2 ± 0.5	2 ± 0.7
D. M. P.	3 ± 0.4	4 ± 1	4 ± 1	9 ± 1.3
D. E. T.	2 ± 0.1	6 ± 0.8	6 ± 1.4	6 ± 0.8
Indalone	4 ± 1	5 ± 1.2	6 ± 1.4	5 ± 1.3
Rutger's 612	2 ± 0.5	5 ± 0.4	8 ± 1.3	5 ± 1

Experiment 3

According to Kalmus and Hocking (1960, p. 8), when mosquitoes were centrifuged in a 9 cm petri dish at 390 rpm and observed under stroboscopic illumination, they were found facing towards the center of the dish, and sometimes walking towards it.

In this study of the same behavior in relation to repellents a plastic petri dish of 9 cm diameter was lined with filter paper on which one radius was drawn in ink. Its lid was extensively perforated by small holes. Mosquitoes, both males and females (50 to 60 adults) were released in this dish and centrifuged at 390 rpm on a turntable and observed under stroboscopic illumination. Mosquitoes were seen as reported by Kalmus and Hocking (1960) facing towards the center and walking towards it. Most of them collected near the center roughly 1 to 1.5 cm from it; fewer mosquitoes remained at the periphery. The centrifugal force at 1 cm from center was 1.7 g and 1.5 cm 2.5 g. As the dish continued to rotate more mosquitoes could be seen moving towards the center. For experiments with repellents the mosquitoes were taken in batches of 15, in a sucking tube, chilled for 1.5 minutes at $14^{o}F$ and then their mesonota painted with repellent on a cold petri dish under a binocular microscope. All four repellents were tested. After treatment the mosquitoes were released in a cage and allowed to recover. They were then introduced in the petri dish (50-60 of them) and made to rotate.

Under stroboscopic illumination it was observed that the mosquitoes did not collect in greater numbers near the center of the dish and the movement towards the center was less noticeable. The dish gave an appearance of a scattered distribution of mosquitoes as compared to a circular distribution near the center in the control. Quite a few (10-15%) faced directions other than the center.

TABLE 11 - Root mean square of angles of inclination of the body axes of *A. aegypti* to the vertical in the presence of repellents in a rotated petri dish in degrees. Means of five replicates ± standard errors.

	Control	Repellents D.M.P.	D.E.T.	Rutger's 612	Indalone
Angle in degrees	0	43 ± 8.4	47 ± 13.3	50 ± 5.4	47 ± 13.3

In another experiment the mosquitoes themselves were not treated but a disc of 4 cm diameter (centrifugal force 3.5 g) was painted with repellent in the center of the dish. Mosquitoes (50-60) were introduced in the dish which was then rotated. It was observed that with an exception of one or two the mosquitoes remained outside the disc, sometimes facing towards it and sometimes turning away from it or walking around it. In yet another experiment when the diameter of the circle painted with repellent was increased to 6 cm (centrifugal force about 5 g) in 9 cm petri dish the same behavior was observed. Most of the mosquitoes remained outside the circle, although the non-treated peripheral belt around the repellent coated circle was only 1.5 cm wide.

Visual Responses

The optomotor and visual responses of mosquitoes have been studied by many workers. Kalmus (1958) reported that *A. aegypti* shows responses to the rotation of the plane of polarization of light. In a later study Kalmus and Hocking (1960, p. 19) observed swarming flight in *A. aegypti* close underneath a weak light source placed on top of a darkened cage, but the same was not observed when a much stronger light was made to pass through a red filter. Mosquitoes were also observed by these workers to aggregate near the margins of black objects when these were placed on top of a weakly illuminated cage.

The visual response of mosquitoes was also studied by Kennedy (1939) and Rao (1947). Kennedy reported that suspended mosquitoes orientated accurately towards a vertical black stripe on a white background. Presented with two stripes the mosquitoes faced one or the other stripe and not between the two. Rao (1947) reported similar findings with *Anopheles maculipennis atroparvus* van Thiel, and *Culex (Culex) molestus* Forskal rendered flightless by the removal of the wings or by sticking them together.

To test the effect of repellents on the visual response of *Aedes aegypti* to black stripes, 20 female mosquitoes were taken in a glass bottle 12 cm tall and with a diameter of 3 cm. The inside of the bottle was lined with white nylon net to give the mosquitoes a good foothold. This bottle was placed inside a glass cylinder 14 cm high and with a diameter of 6 cm. The bottle and the cylinder were placed on a thick glass plate which was resting on a tripod stand. Under the glass was placed a 40 watt electric lamp which was covered all around with a cylinder of black paper so that light could go only upwards and light the bottle and the cylinder outside it uniformly from inside. In order that the inside of the cylinder be evenly illuminated, a filter paper was placed on the glass plate on which the outer cylinder and the inner bottle rested. The outer cylinder was divided into four quadrants and the alternate two quadrants were covered with black paper strips, each covering 90°. The remaining two quadrants were left uncovered, (figure 2).

As the outer cylinder was placed around the inner bottle containing mosquitoes and kept there for a short time, the mosquitoes inside moved and came to rest on the wall of the bottle facing the black stripes. The outer cylinder was then rotated 90° so that all the mosquitoes now faced uncovered portions of the cylinder. The mosquitoes moved again in the direction of the black stripes and again came to rest opposite to them. This behavior could be observed again and again. However, when the antennae were painted with any of the four repellents they showed complete indifference to the black stripes and did not move towards them as in the control.

The experimental data on the effects of repellents on **behaviour** are summarized in table 11A.

TABLE 11A - Summary of data on the effect of repellents on responses to stimuli.

Table / Page	Response	Effect
3/12 & 4/13	Sugar feeding	Inhibition
5 / 15	Mating	Partial Inhibition
6/18 & 7/19	Oviposition-site treated -tarsi treated	Inhibition No Inhibition
8/20 & 9/21	To wind	Partial Inhibition (D. E. T. only)
10/23 & 11/24	Gravity	Inhibition
/ 24	Optomotor	Inhibition

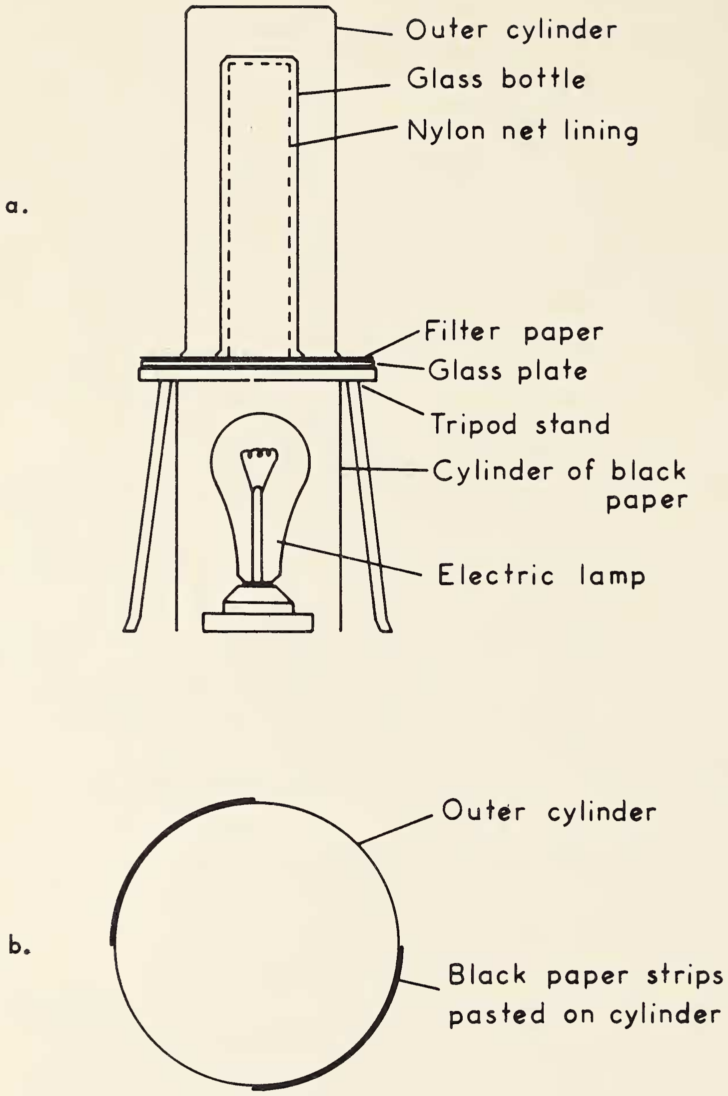


Figure 2. Diagrams showing: (a) a vertical section of the apparatus used for testing the visual response of *A. aegypti* females to black stripes in relation to repellents, and (b) a cross section of the outer cylinder.

EXPERIMENTAL - LIPOID SOLVENTS

Amongst the advocates of chemical theories referred to in a previous section, many have suggested lipoid solubility as a basis of olfaction (Cohn, 1924; Dyson, 1938; Dethier & Chadwick, 1947; Dethier, 1948). Experiments were conducted to examine the effect of fat solvents applied on the antennal chemoreceptors of *Aedes aegypti* females on their behavior towards a host.

Ten female *Aedes aegypti* eleven days old were taken for each experiment. The mosquitoes, which were fed on sugar solution only, were taken in a sucking tube and chilled for 1.5 minutes at 15°F. They were then placed on a filter paper on top of a cold petri dish and their antennae were washed with lipid solvents applied with a fine camel hair brush. The mosquitoes were then transferred to a clean petri dish lined with filter paper in a one cubic foot cage and allowed to recover. Thirty minutes after the operation a hand was introduced into the cage and the number of landings of mosquitoes on it was recorded for a period of 15 minutes. Mosquitoes were shaken off gently on landing and were not allowed to feed on blood. The antennae of controls were rubbed with a clean dry brush.

The observations are recorded in table 12, and show that the number of landings decreased very significantly on washing the antennal chemoreceptors with the lipid solvents. But whether the decrease in landings is due to the loss of lipids from the chemoreceptors, or due to the narcotic, anesthetic, or other effect of the solvents is uncertain.

TABLE 12 - Numbers of *Aedes aegypti* females landing on a hand in a 15 minute period after treatment of the antennae with lipoid solvents. Means of three replicates ± standard errors.

Control	Acetone	Ether
174 ±8	52 ±12	43 ± 13

DISCUSSION

The action of repellent chemicals on mosquitoes has no specificity for blood feeding behavior. It has been shown that repellents in the vapor phase have the following effects on *Aedes aegypti*. They inhibit feeding on both blood and sugars, reduce the mating rate, and cause rejection of oviposition sites. The repellents also affected orientation to gravity and centrifugal force and the visual response to black stripes.

Mosquitoes became quiescent and less active when repellents were applied on them. This slowing down of motor activity suggests the external stimuli normally acting on the mosquito are perhaps blocked or interfered with by the repellent. As there is no delay in the effect of repellents on the behavior of mosquitoes, that is, protection is obtained immediately these materials are applied, their action on the insect may be assumed to occur at the surface of the body. Repellents have not been shown to penetrate rapidly into the body where they could act on the nerve synapses or the central nervous system, nor have they been shown to affect the muscular system directly. It thus seems unlikely that they act by blocking the nerve impulses or the motor response. The most probable action seems therefore to be the blocking of reception of stimuli at the receptor site.

Somewhat different behavior in relation to repellents of another kind has been described by Kennedy (1947). He studied the effects of contact with DDT on the activity and distribution of mosquitoes. He argued from his experiments that a variety of reactions may give rise to repulsion. Reactions may occur at a distance or only after contact with a repellent surface. The contact stimuli may be mechanical or chemical. The reactions may take the form of an increase of merely random activity or they may be directed away from the surface. They may be quick or slow to appear and weak or strong in expression. In contrast to my findings of reduced activity in his work an increase in activity was found.

The factors that attract mosquitoes to the host have been reviewed above. The mode of action of insect repellents can be best understood when studied in relation to these factors.

The effects of repellents on the evolution of carbon dioxide and moisture from a human arm, and the correlation of this evolution with the natural attractiveness of human beings and protection time of repellents were studied by Gouck and Bowman (1959) at Orlando, Florida. In their experiments, repellents applied to the arms of three subjects reduced the CO_2 emitted by 9 to 14 per cent but they concluded: "Although these reductions are considerably greater than the differences between untreated arms (4%) they are not great enough to indicate that the mode of action of these repellents is based upon the retardation of carbon dioxide evolution". The repellents used were, dimethyl phthalate, diethyl toluamide and ethyl hexanediol. With regard to the moisture collected from untreated and repellent treated arms they concluded: "The quantities from the arms of all subjects varied from day to day but in most individual tests the two arms agreed within about 5 per cent indicating that no real difference in the amount of moisture evolved was caused by application of repellents." They believed that the protection time is governed by the rate of loss of repellent from the skin by absorption and evaporation. Peters and Kemper (1958) have shown that there are no considerable temperature differences between repellent treated and untreated parts of the skin.

In the light of these findings it can be said that repellents affect the reception of these stimuli rather than the stimuli themselves. This supports the hypothesis advanced that repellents affect many kinds of behavior of mosquitoes by interfering in the reception of many different kinds of stimuli.

Search for chemical factors other than carbon dioxide attracting mosquitoes to the host has claimed the attention of many workers. The findings of Shaerffenberg and Kupka (1951) and Burgess and Brown (1957) have indicated that attractive factors other than carbon dioxide are present in the vapor from mammalian blood and body exudations. A distillate obtained from mammalian blood by Shaerffenberg and Kupka

(1959) proved highly attractive to *Culex pipiens* L. Rudolfs (1922) found benzoic acid, dilute ammonia, phenylalanine, alanine, aspartic acid, cystine, and hemoglobin to be attractive to *Aedes sollicitans* Walker and *Aedes cantator* Coquillett, but Reuter (1936) found the last six materials unattractive to *Anopheles maculipennis atroparvus*. Brown and Carmichael (1961) reported that L-lysine and L-alanine were attractive to *Aedes aegypti.* The effect of repellents in association with these chemicals found to be attractive remains to be studied.

Travis and Smith (1951) evaluated dimethyl phthalate, indalone, and ethyl hexanediol against *Aedes aegypti* besides other mosquitoes, and found average repellent times (i. e., times in minutes from application of the repellent to the first bite) as follows: ethyl hexanediol - 331 minutes, dimethyl phthalate - 247 minutes, and indalone - 111 minutes. Although the results of my experiments are not strictly comparable with those of Travis and Smith (1951) for I worked with a different culture of mosquitoes and at a different time and place, the mosquitoes fed on blood much sooner after treatment when repellents were applied on the mosquito receptor sites. For example, about 33 per cent of mosquitoes fed on blood within 10 minutes after application of dimethyl phthalate on both antennae. When diethyl toluamide, indalone, and ethyl hexanediol were separately applied on both antennae, some of the first bites were recorded after 10 minutes. The reason for this behavior is perhaps the more rapid adaptation of the receptors to the repellents because of the greater concentration gradient resulting from their application on the receptors themselves. In this way the threshold for reception of repellents increased greatly but that for other stimuli remained the same. The sequence of stimuli and responses leading to blood feeding therefore remained unaffected. But this is, of course, incompatible with the hypothesis that repellents block all receptors.

The presence of separate chemoreceptor neurons mediating acceptance and rejection is assumed from the study of labellar chemoreceptor cells of *Phormia regina*. These cells have been the subject of coordinated behavioral, histological, and physiological study. A chemosensory hair of the labellum of this blowfly was described by Dethier (1955) as a hollow extension of the body cuticle possessing two distinct lumina. The chemosensory hair has been shown to be associated with three bipolar neurons, two of which send distal fibers to the terminal papilla by way of the thick-walled lumen of the hair. Dethier (1955) concluded that one of these neurons mediates acceptance while the other mediates rejection. On electrophysiological studies one of the two neurons was later designated the L fiber (for large spikes which responded to salts and the other the S fiber (for small spikes) which responded to sugars (Hodgson *et al.* 1955; Hodgson and Roeder, 1956). Wolbarsht and Dethier (1958) were able to detect the spikes of the third neuron which terminated in a process at the base of the hair. It was designated M for mechanoreceptor. Evans and Mellon (1962) have now detected spikes from a fourth neuron which responds to water.

In the course of electrophysiological studies of chemoreceptor hairs it has been shown that when mixed stimuli are applied there is an interaction between activity in the L and S fibers (Hodgson, 1956 , 1957; Morita, 1959; Sturckow, 1959). Hodgson (1957) found that the presence of S impulses is accompanied by a decrease in L impulses and conversely the S spikes decrease when the L fiber is stimulated. My experiments show that the repellents block the reception of attractant and other stimuli. This assertion needs to be confirmed by electrophysiological methods.

Mosquitoes with antennae painted with diethyl toluamide landed, walked around, and even probed on an arm also treated with the same repellent but did not feed on blood. This may be explained in one of two ways. It may be that the piercing of the skin by the mosquito is induced by some chemical factor on the skin which was neutralized by the application of the repellent or, it may be due to the effect of repellent on the action of thermoreceptors or contact chemorecptors which induce feeding on blood. The latter explanation would be more in conformity with the findings that repellents interfere with the reception of all kinds of stimuli affecting the total behavior of mosquitoes.

The study on blood feeding when repellents were applied on parts of the mosquito revealed that of the four repellents dimethyl phthalate has the greatest effect on blood feeding behavior when it is painted on the tarsal receptors and the smallest effect when it is painted on the receptors of the antennae. As is known, the olfactory receptors are located on the antennae and the contact chemoreceptors mostly on the tarsi of the mosquito. Dimethyl phthalate, which has the highest boiling point and hence the lowest vapor pressure, may, for this reason, have more effect than the other repellents through the tarsal chemoreceptors in the liquid phase but less than these through the olfactory receptors of the antennae where it has to act in the vapour phase which is at a lower concentration.

That repellents also acted as irritants was evident from the intense wriggling activity of the mosquito when repellents were applied on the proboscis and from the vigorous cleaning of repellent from the antennae with the tarsi of the fore legs. This evident awareness of the presence of an irritant chemical indicates the existence of receptors sensitive to it, perhaps those of the common chemical sense. It may well be that these are the only receptors not blocked by repellents.

In the vapor phase repellents were found to inhibit landing of mosquitoes. This was observed in experiments on blood feeding, sugar feeding, oviposition, and air flow. In the liquid phase, however, the repellents showed more irritant and some toxic effects, and the mosquitoes showed considerable decrease in locomotor activity, in part on account of preoccupation with attempts at cleaning off the repellents.

Repellents have been defined as compounds which elicit an avoiding reaction (Dethier, 1956b). While the four materials studied may all do this, this is by no means their only effect and may not, indeed, be the most important one.

ACKNOWLEDGEMENTS

I am most thankful to Professor B. Hocking for his keen interest, constructive criticism and many valuable suggestions during the progress of this work. I also gratefully acknowledge the support of the Defence Research Board, Department of National Defence, Government of Canada, who financed this study.

REFERENCES

Amoore, J. E. 1963. Stereochemical theory of olfaction. Nature, Lond. 198 : 271 - 272.

Amoore, J. E., Johnston, J. W. Jr., and Rubin, M. 1964. The stereochemical theory of odor. Sci. Amer. 210 (2) : 42 - 49.

Banks, C. S. 1908. Biology of Philippine Culicidae. Philip. J. Sci. 3 (A) 235 - 258.

Bar Zeev, M. 1960. The reaction of mosquitoes to moisture and high humidity. Ent. exp. appl. 3 : 198 - 211.

Bates, M. 1949. The Natural History of Mosquitoes. Macmillan, New York. XV. 379 pp.

Bishop, A., and Gilchrist, B. M. 1949. Experiments upon the feeding of *Aedes aegypti* through animal membranes, etc. Parasitology 37 : 85 - 100.

Brown, A. W. A. 1951. Studies on the responses of the female *Aedes* mosquito. Part IV. Field experiments on Canadian species. Bull. ent. Res. 42 : 575 - 582.

Brown, A. W. A. 1956. Factors which attract *Aedes* mosquitoes to humans. Proc. tenth int. Congr. Ent. 3: 757 - 763.

Brown, A. W. A., and Carmichael, A. G. 1961. Lysine and alanine as mosquito attractants. J. econ. Ent. 54 (2) : 317 - 324.

Browne, B. L. 1960. The role of olfaction in the stimulation of oviposition in the blowfly, *Phormia regina*. J. Insect Physiol. 5 : 16 - 22.

Burgess, L., and Brown, A. W. A. 1957. Studies on the responses of the female *Aedes* mosquito. Part VIII. The attractiveness of beef blood to *Aedes aegypti* L. Bull. ent. Res. 48 : 783 - 793.

Christophers, S. R. 1960. *Aedes aegypti*, the yellow fever mosquito - Its life history, bionomics and structure. Cambridge University Press. 739 pp.

Cohn, G. 1924. Die Riechstoffe. 2nd Ed. Braunschweig: Verlag Vieweg. pp. viii + 216.

Davies, J. T. 1962. The mechanism of olfaction. p. 170-179, Symposia of the Society for Experimental Biology. No. XVI - Biological receptor mechanisms. Cambridge University Press.

DeLong, D. M. 1946. Insect behaviour - Mosquito attractancy and repellency. U. S. Natl. Res. Council, Insect Control Committee Abstr. Bull. n. s. 11, pp. 515 - 520.

DeLong, D. M., Davidson, R. H., Peffly, R. L., and Venard, C. E. 1945. A study of the action of insect repellents in terms of their effect on insect behaviour and in relation to their properties. U. S. Natl. Res. Council, Insect Control Committee. Abstr. Bull. n. s. 3, pp. 96 - 102.

Dethier, V. G. 1948. Chemoreceptors in insects. Physiol. Rev. 28 : 220 - 254.

Dethier, V. G. 1952. The relation between olfactory response and receptor population in the blowfly. Biol. Bull. Wood's Hole 102 : 111 - 117.

Dethier, V. G. 1955. The physiology and histology of the contact chemoreceptors of the blowfly. Quart. Rev. Biol. 30: 348 - 371.

Dethier, V. G. 1956a. Attractants and repellents. 43rd Annual Meeting Proceedings of the Chemical Specialties Manufacturers Association Inc. 1 - 8.

Dethier, V. G. 1956b. Repellents. Annu. Rev. Ent. 1 : 181 - 202.

Dethier, V. G. 1957. Chemoreception and the behaviour of insects. Survey Biol. Progr., Academic Press, N. Y. 3 : 149 - 183.

Dethier, V. G., and Chadwick, L. E. 1947. Rejection thresholds of the blowfly for a series of aliphatic alcohols. J. gen. Physiol. 30 (3) : 247 - 253.

Dethier, V. G., and Yost, M. T. 1952. Olfactory stimulation of blowflies by homologous alcohols. J. gen. Physiol. 35 (6) : 823-839.

Dyson, G. M. 1938. The scientific basis of odor. Chem. & Ind. (Rev.) 57 : 647 - 651.

Evans, D. R. 1961. The effect of odours on ingestion by the blowfly. J. Insect Physiol. 7 : 299 - 304.

Evans, D. R. and Mellon, De F. 1962. Electrophysiological studies of a water receptor associated with the taste sensilla of the blowfly. J. gen. Physiol. 45 : 487 - 500.

Frings, H. and Hamrum, C. L. 1950. The contact chemoreceptors of adult yellow fever mosquitoes, *Aedes aegypti*. J. N. Y. ent. Soc. 58 : 133 - 142.

Goeldi, E. A. 1905. Os mosquitos no Para Reuniae gutro trabalhos sobre os mosquitos indigenas, principalmente as aspecies que molestam homen. Mem. Mus. Goeldi Hist. nat. Ethnogr. 4 : 154 pp.

Gordon, R. M. and Lumsden, W. H. R. 1939. A study of the behaviour of the mouth parts of mosquitoes when taking up food from living tissue, etc. Ann. trop. Med. Parasit. 33 : 259 - 278.

Gouck, H. K., and Bowman, M. C. 1959. Effect of repellents on the evolution of CO_2 and moisture from human arms. J. econ. Ent. 52 (6) : 1157 - 1159.

Haddow, A. J. 1942. The mosquito fauna and climate of native huts at Kisumu, Kenya. Bull. ent. Res. 33 : 91 - 142.

Headlee, T. J. 1941. New Jersey mosquito problems. Proc. New Jersey Mosquito Exterm. Assoc. 28 : 7 - 12.

Hocking, B. 1952. Protection from northern biting flies. Mosquito News. 12 : 91 - 102.

Hodgson, E. S. 1956 . Physiology of the labellar sugar receptors of flies. Anat. Rec. 125, 555.

Hodgson, E.S. 1957. Electrophysiological studies of arthropod chemoreception. 11. Responses of labellar chemoreceptors of the blowfly to stimulation by carbohydrates. J. Insect Physiol. 1 : 240 - 247.

Hodgson, E.S., Lettvin, J.Y., and Roeder, K.D. 1955. Physiology of a primary chemoreceptor unit. Science 122 : 417 - 418.

Hodgson, E.S., and Roeder, K.D. 1956. Electrophysiological studies of arthropod chemoreception. 1. General properties of the labellar chemoreceptors of Diptera. J. cell. comp. Physiol. 48 : 51 - 76.

Howlett, F.M. 1910. The influence of temperature upon the biting of mosquitoes. Parasitology 2 : 479 - 484.

Huffaker, C.B., and Back, R.C. 1943. A study of methods of sampling mosquito populations. J. econ. Ent. 36 : 561 - 569.

Jones, F.N., and Jones, M.H. 1953. Modern theories of olfaction: a critical review. J. Psychol. 36 : 207 - 241.

Kalmus, H. 1958. Responses of insects to polarized light in the presence of dark reflecting surfaces. Nature, Lond. 182 : 1526 - 1527.

Kalmus, H. and Hocking, B. 1960. Behaviour of *Aedes* mosquitoes in relation to blood feeding and repellents. Ent. exp. & appl. 3 : 1 - 26.

Kellogg, F.E. and Wright, R.H. 1957. The guidance of flying insects. V. Mosquito attraction. Canad. Ent. 94 (10) : 1016.

Kennedy, J.S. 1939. The visual responses of flying mosquitoes. Proc. zool. Soc. Lond. (A). 109 : 221 - 242.

Kennedy, J.S. 1947. The excitant and repellent effects on mosquitoes of sub-lethal contacts with DDT. Bull. ent. Res. 37 : 593 - 607.

Macfie, J.W.S. 1915. Observation on the bionomics of *Stegomyia fasciata* Bull. ent. Res. 6 : 205 - 229.

Morita, H. 1959. Initiation of spike potentials in contact chemosensory hairs of insects. III. D.C. stimulation and generator potential of labellar chemoreceptor of Calliphora. J. cell. comp. Physiol. 54 : 189 - 204.

Parker, A.H. 1948. Stimuli involved in the attraction of *Aedes aegypti* L. to man. Bull. ent. Res. 39 : 387 - 397.

Peters, W. 1956. Experimentelle Untersuchungen zur Wirkungsweise Insectenabwehrender Mittel. Z. angew. Zool. 1 : 1 - 75.

Peters, W. and Kemper, H. 1958. Über die Wirkungsweise von Repellents. Ein Beitrag sum Problem der Wirtsfindung die blutsaugenden Arthropoden. Proc. tenth int. Congr. Ent. 2 : 389 - 392.

Rahm, U. 1956. Zum Problem der Attraction von Stechmücken durch den Menschen. Acta trop. 13 (4) : 319 - 344.

Rahm, U. 1957. Zur Bedeutung des Duftes und des Schweisses bei der Attraktion von *Aedes aegypti* durch Menschen. Acta. trop. 14 (3) : 208 - 217.

Rahm, U. 1958. Die attraktive Wirkung der von Menschen abgegebenen Duftstoffe auf *Aedes aegypti* L. Z. Tropenmed. u. Parasit. 9 : 146-156.

Rahm, U. 1958. Die Funktion der Antennen, Palpen und Tarsen von *Aedes aegypti* L. beim Aufsuchen des Wirtes. Rev. suisse Zool. 65 : 779 - 792.

Rao, T.R. 1947. Visual responses of mosquitoes artificially rendered flightless. J. exp. Biol. 24 : 64 - 78.

Reuter, J. 1936. Orienterend ondersoek naar de oorzaak van het gedrag van *Anopheles masculipennis* Meigen by de voedselkeuze. Acad. Proefachr. Rijksuniv. Leiden, 118 pp.

Richards, O. W. 1927. Sexual selection and allied problems in the insects. Camb. phil.Soc.Rev. & Proc. 2 : 298 - 360.

Roth, L.M. 1948. A study of mosquito behaviour. An experimental study of the sexual behaviour of *Aedes aegypti* L. Amer. midl. Nat. 40 : 265 - 352.

Roth, L.M. 1951. Loci of sensory end organs used by mosquitoes (*A. aegypti* L. and *Anopheles quadrimaculatus* Say.) in receiving host stimuli. Ann. ent. Soc. Amer. 44 : 59 - 74.

Roth, L.M. and Willis, E.R. 1952. Possible hygroreceptors in *Aedes aegypti* L. and *Blatella germanica* L. J. Morph. 91 : 1-14.

Ross, R. 1898. Report on the cultivation of *Proteosoma* Labbé in grey Mosquitoes. Indian med. Gaz. 33 : 173-5, 401-8, 448-51.

Rudolfs, W. 1922. Chemotropism of mosquitoes. New Jersey Agric. Exp. Sta. Bull. 367.

Shaerffenberg, B. and Kupka, H. 1951. Untersuchungen über die geruchliche blutsaugende Insekten. 1. Über die Wirkung eines Blutduftstoffes auf *Stomoxys* und *Culex*. Öst. zool. Z. 3: 410 - 424.

Shaerffenberg, B. and Kupka, H. 1959. Der attraktiv Faktor des Blutes für blutsaugende Insekten. Naturwissenschaften 46: 457 - 458.

Shannon, R.C. and Putnam, P. 1934. The biology of *Stegomyia* under laboratory conditions. I and II. Proc. ent. Soc. Wash. 36 : 185 - 242.

Slifer, E.H. 1962. Sensory hairs with permeable tips on the tarsi of the yellow fever mosquito, *Aedes aegypti*. Ann. ent. Soc. Amer.. 55 : 531 - 535.

Slifer, E.H. and Sekhon, S.S. 1962. The fine structure of the sense organs on the antennal flagellum of the yellow fever mosquito, *Aedes aegypti* L. J. Morph. 111 (1) : 49 - 68.

Steward, C.C. and Atwood, C.E. 1963. The sensory organs of the mosquito antenna. Canad. J. Zool. 41 (4) : 577 - 594.

Sturckow, B. 1959. Über den Geschmackssinn und den Tastsinn von *Leptinotarsa decemlineata* Say. (Chrysomelidae). Z. vergl. Physiol. 42 : 255 - 302.

Thiel, P.H. Van. 1937. Quelles sont les excitations incitant l'*Anopheles maculipennis* atroparvus à visiter et à piquer l'homme ou le bétail? Bull. soc. Pat. exot. 30 : 193 - 203.

Thiel, P.H. Van., and Laarman, J.J. 1953. What are the reactions by which the female *Anopheles* finds its blood supplier? Docum. Med. geogr. trop. 6 : 156 - 161. Idem Acta leidensia. 1954. 24 : 180 - 187.

Thompson, R. P. and Brown, A. W. A. 1955. The attractiveness of human sweat to mosquitoes and the role of carbon dioxide. Mosquito News. 15 (2) : 80 - 84.

Travis, B. V. and Smith, C. N. 1951 Mosquito repellents selected for use on man. J. econ. Ent. 44 (3) : 428 - 429.

Wallis, R. C. 1954. A study of oviposition activity of mosquitoes. Amer. J. Hyg. 60: 135 - 168.

Wiesmann, R., and Lotmar, R. 1949. Beobachtungen und Untersuchungen uber den Wirkungsbereich des neuen Repellent Kik-Geigy. Acta Trop. 6: 135 - 168.

Willis, E. R. 1947. The olfactory responses of female mosquitoes. J. econ. Ent. 40 : 769 - 778.

Wolbarsht, M. L. and Dethier, V. G. 1958. Electrical activity in the chemoreceptors of the blowfly. 1. Responses to chemical and mechanical stimulation. J. gen. Physiol. 42 : 393 - 412.

Wright, R. H. 1962. Studies in mosquito attraction and repulsion. V. Physical theory. Canad. Ent. 94 : 1022 - 1029.